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Visual Evidence Demonstrating the Activity of the Two-Phase Mouthwash

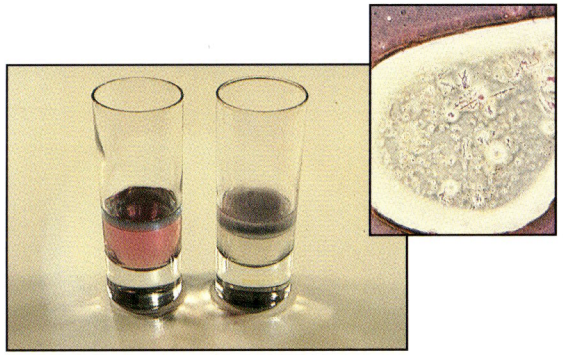

Figure 3

Figure 4

Figure 3 shows the two-phase mouthwash Assuta™ before use (glass on left) and following expectorations (glass on right). The food color in the lower aqueous phase is also a biological stain. Bacteria and debris, bound to the droplets, absorb the stain with a corresponding loss of color in the bottom phase. Microscopic examination (Figure 4) reveals hundreds of bacteria, bound to a single oil droplet.

Advantages of The Two-Phase Mouthwash

1. Scientifically proven ability to reduce oral-malodor related parameters for long periods.
2. Superior ability to desorb bacteria.
3. The only mouthwash whose activity can actually be demonstrated in the customer's home.
4. Long lasting fresh taste with no alcoholic burning or oily feel.
5. Fluoride has been incorporated into the formulation for caries prevention.

References:

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3. Weiss, E., M. Rosenberg, H. Judes and E. Rosenberg. Cell surface hydrophobicity of adherent oral bacteria. **Current Microbiology** 7:125-128, 1982.
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5. Rosenberg, M. Basic and applied aspects of microbial adhesion at the oil:water interface. **CRC Crit Rev Microbiol** 18:159-173, 1991.
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7. Rosenberg, M., H. Judes and E. Weiss. Desorption of adherent bacteria from a solid hydrophobic surface by oil. **J Microbiol Methods** 1:239-244, 1983.
8. Goldberg, S., Y. Konis and M. Rosenberg. Effect of cetylpyridinium chloride on microbial adhesion to hexadecane. **Appl Environ Microbiol** 56:1678-1682, 1990
9. Goldberg, S. and M. Rosenberg. Bacterial desorption by commercial mouthwashes vs two-phase oil:water formulations. **Biofouling** 3:193-198, 1991.
10. Rosenberg, M., I. Gelernter, M. Barki and R. Bar-Ness. Daylong reduction of oral malodor by a two-phase oil:water mouthrinse, as compared to chlorhexidine and placebo rinses. **J Periodontol** 63:39-43, 1992.
11. Yaegaki, K. and Sanada, K. 1992. Effects of a two-phase oil-water mouthwash on halitosis. **Clin Prevent Dent** 14:5-9, 1992.

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A Scientific Advance In Oral Hygiene

ASSUTA

Two-Phase Mouthwash For Long Lasting Reduction In Oral Malodor

TID
Shemen Industries Ltd.

Background

Mouthrinsing is a common oral hygiene practice dating back to ancient times. One of the major concerns which leads to frequent use of mouthrinses is halitosis (badbreath) (1,2). Bad breath usually originates within the oral cavity itself, due to production of gases (primarily volatile sulfur compounds, VSC) by sequestered deposits of microorganisms, generally under anaerobic conditions. Periodontal diseases commonly result in bad breath. Other loci of oral microbial putrefaction include the tongue dorsum, areas of food impaction, and improper dental restorations.

The efficacy of current commercial mouthwashes is based primarily on the antimicrobial activity of their components. We have developed a novel two-phase mouthwash **which also detaches and removes bacteria and the debris which they feed upon from the oral cavity.** This is based upon the enhanced ability of the oil droplets to bind oral microorganisms and debris. Metabolism and activity of bacteria which are not removed during the rinse are inhibited by antibacterial agents in both the aqueous and oil phases.

Scientific Support

In 1982, Weiss *et al.* (3) reported that a high proportion of adherent oral microbial isolates adhere to hexadecane. In 1983, Rosenberg *et al.* demonstrated that washed, dispersed suspensions of microorganisms obtained directly from supragingival dental plaque adhere in high proportions to test hydrocarbons (4). These initial observations led to the idea of using two-phase oil:water mouthwashes for *in vivo* desorption of oral microbiota. Subsequent studies have demonstrated that a wide variety of laboratory strains of oral microorganisms, including *Actinobacillus actinomycetemcomitans, Actinomyces viscosus* and *A. naeslundii, Bacteroides(Porphyromonas) gingivalis, Candida albicans, Streptococcus salivarius, Streptococcus sanguis* and certain *Streptococcus mutans* strains, adhere with high affinity to hydrocarbons (5,6). Initial experiments showed that microorganisms which adhere to hydrocarbons, also bind to various nontoxic oils (7). This suggested that two-phase, oil:water mouthrinses could be of clinical value in desorbing oral microorganisms. *In vitro* experiments showed that **aqueous:oil combinations removed over 97% of a film** of *Acinetobacter calcoaceticus* RAG-1 cells onpolystyrene cuvettes (7).

It was subsequently found that adhesion of oral microorganisms to oil could be further enhanced by low concentrations of a cationic antibacterial agent (cetylpyridinium chloride) which is commonly added to mouthwashes (8). Prototype formulations, consisting of an aqueous phase containing CPC, a food color, and sweetener, and an oil phase comprising a mixture of vegetable and essential oils, were developed. **Such formulations maintained dramatic in vitro bacterial desorption properties (9),removing 92-98% of a bound bacterial layer, as compared to 15-50% for various commercial formulations** (see Figures 1 and 2).

Microbial desorption by two-phase mouthwash

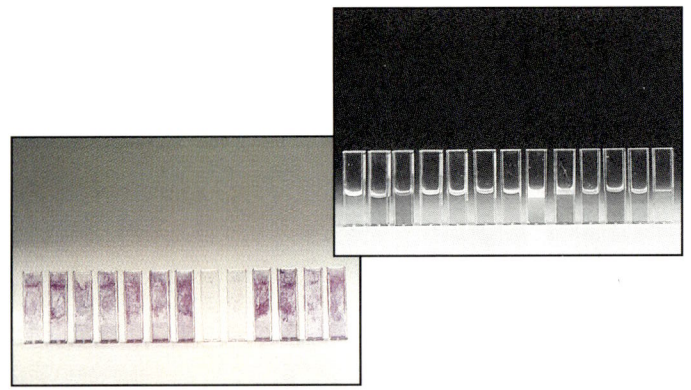

Figure 1

Figure 2

In comparison to leading commercial mouthrinses, two-phase oil-water formulations (fifth and sixth cuvettes from left) almost completely desorbed the bacterial layer in a published in vitro experiment (9). Figure 1 shows the bacteria-coated cuvettes prior to mixing. Figure 2 showes the cuvettes following mixing and staining with gentian violet to visualize the residual microbial layer.

Two recently published studies attest to the malodor reducing properties of two-phase formulations. In the first study (10), the daylong anti-odor and antibacterial properties of the two-phase mouthwash were compared with placebo. **The two-phase mouthwash was effective 8-10 hours following use**: on an individually-computed basis, there was a 51% and 50% mean reduction in organoleptic scores and sulphide levels, respectively, following use of the two-phase mouthwash as compared to the placebo.

In an independent study published in 1992 by Japanese researchers, **the two-phase mouthwash led to a reduction of 80% in volatile sulphides, 3.5 hours after use** (11). A commercial Japanese mouthwash showed only a 30% reduction. Furthermore, in vitro results showed complete inhibition of volatile sulphide and 2-ketobutyrate production by the two-phase mouthwash, as well as a 65% decrease in methionine metabolism. The authors concluded that **"the two-phase mouthwash inhibits the production of volatile sulphide"**. The researchers also demonstrated that the oil droplets in strongly the mouthwash can remove not only oral microorganisms, but other sources of malodor, such as degraded epithelial cells.

Another unique feature of the two-phase mouthwash system is that one can actually see it working (2). The aqueous phase contains a food color which is also a biological stain. Following swishing, one can expectorate the emulsion into a glass or test tube. A few minutes later, the phases separate. The biological debris, stained red by the dye, can be observed adhering to the oil droplets. The bottom aqueous phase concomitantly loses its color, which has been adsorbed by the cells adhering to the oil droplets. (see Figures 3 and 4)

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DIASLIDE®

Urine Culture Device

DIASLIDE® - One step ahead for the detection of urinary tract infection

- Isolation and identification of individual colonies in high concentrations of bacteriuria
- Bedside device ideal for immediate sampling to prevent contamination
- A unique design enables testing of minimal amount of urine sample
- See-through casing – easily viewed results
- Simple, clean and rapid protocol
- Unique transport and incubation package

NOVAmed Ltd.

Diaslide® is a superior device designed to be one step ahead of all urine culturing devices.

Diaslide® combines the sensitivity and specificity of the traditional culture media and the convenience of a dipslide, which together with special unique features, makes it the leading device for urinary tract infections testing.

DESCRIPTION:

The Diaslide® device is a small transparent plastic casing with two hinged sections facing each other containing CLED and MacConkey agars. Diaslide®'s agars are elongated in order to ensure longitudinal streaking of urine by a smart "fork"-like sampler. When pulling the sampler through the casing devise each agar surface is streaked by the tip of one of the tips, as well as by the bent "joint" of the other tip. The tip inoculation yields a streaking dilution of several orders of magnitude, whereas inoculation by the "joint" yields a relatively uniform spreading of the sample.
Each Diaslide® is packed in a sterile, flexible transparent plastic packaging, which is tightly reclosed after sampling and during incubation, creating a closed system to eliminate potential contamination from the surroundings.

DIRECTIONS FOR USE:

1.

Remove the Diaslide® from its package, being careful not to touch the sampler tips.

2.

Dip the sampler tips into the urine, making sure that they are immersed up to the point where they meet.

3.

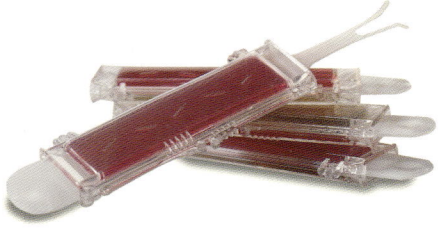
Hold device vertically and use other hand to draw the sampler through the casing in a straight fashion.

Discard the sampler.

4.

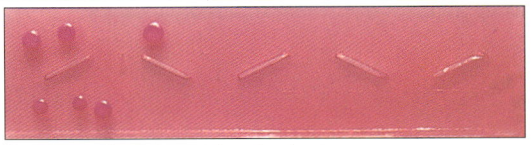
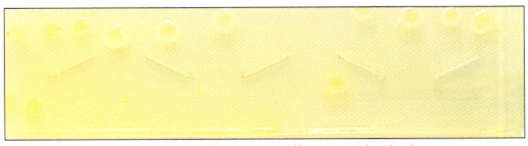
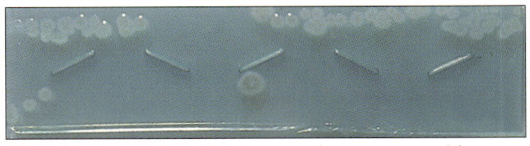
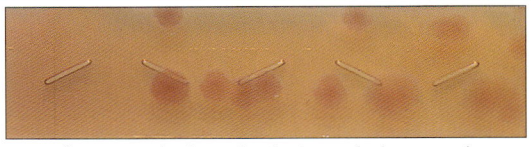
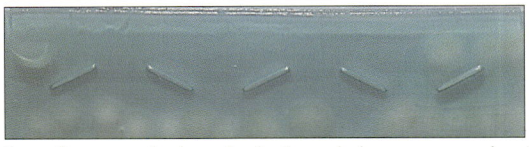
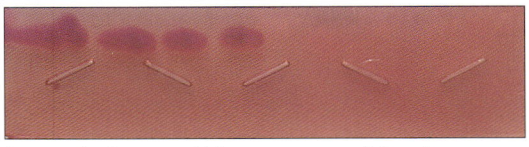
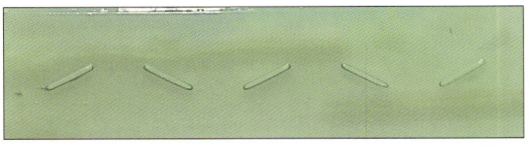
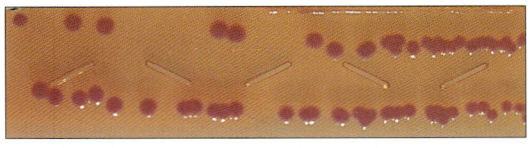
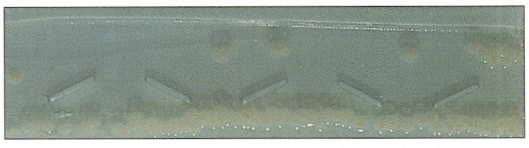
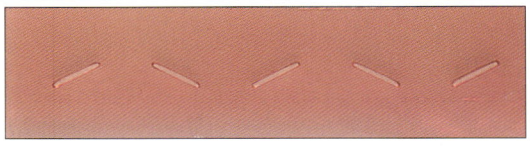
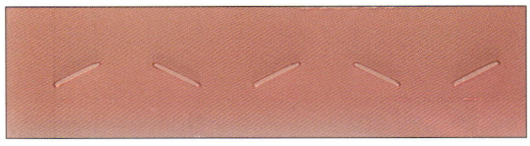
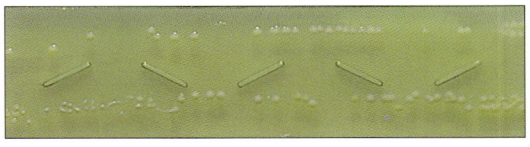
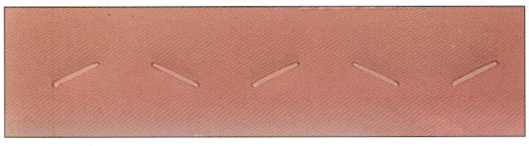
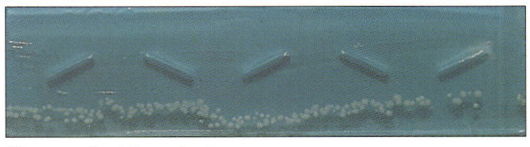
Return the casing into its packaging and incubate vertically at 37°C for 18-24 hours.

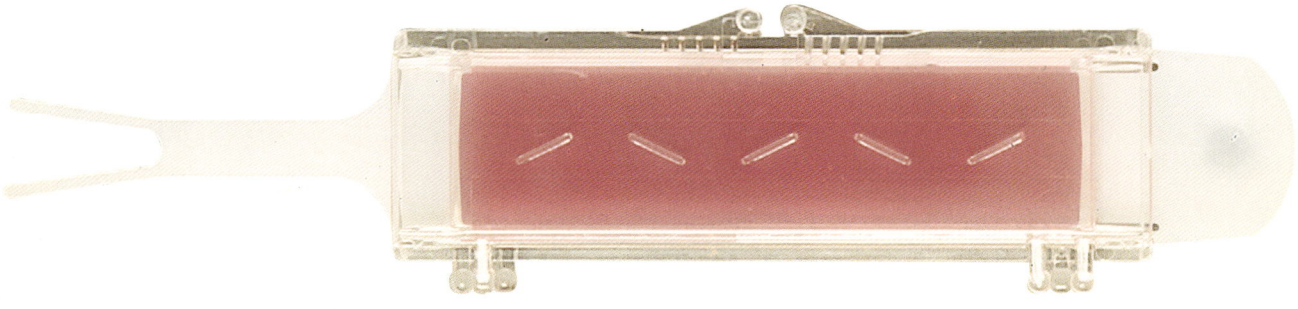
Read and interpret results.



Bacterial Morphology Chart

Typical cultural response on Diaslide® media

	MacConkey agar	CLED agar
Escherichia coli (Gram-negative)	 Round medium size pink-red colonies.	 Round medium size colonies, yellow with darker center.
Proteus mirabilis (Gram-negative)	Round medium size colonies, translucent to light red	 Round medium size colonies, translucent to gray-blue
Pseudomonas aeruginosa (Gram-negative)	 Large flat unevenly shaped colonies, colorless to red	 Large flat unevenly shaped colonies, colorless to green colored
Klebsiella pneumoniae (Gram-negative)	 Large colonies, mucoid in appearance, pink-red	 Large mucoid, colonies, often run together white-to yellow colored
Enterobacter aerogenes (Gram-negative)	 Round medium size colonies, red colored	 Round medium size colonies, white-yellow colored
Enterococcus faecalis (Gram-positive)	 No growth	Small round white-yellow colored colonies
Staphylococcus aureus (Gram-positive)	 No growth	 Round small golden colonies
Candida	 No growth	 Very small white colonies



Bacterial Density Count

The number of colonies grown on both agar surfaces is directly proportional to their concentration in the original urine specimen.
The bacterial concentration of the urine is determined by comparison of the colony density with the reference chart, provided in the instruction manual.
To estimate the bacterial concentration in the urine sample from the number of colonies grown on the Diaslide® we used the following correlation, determined for E.coli:

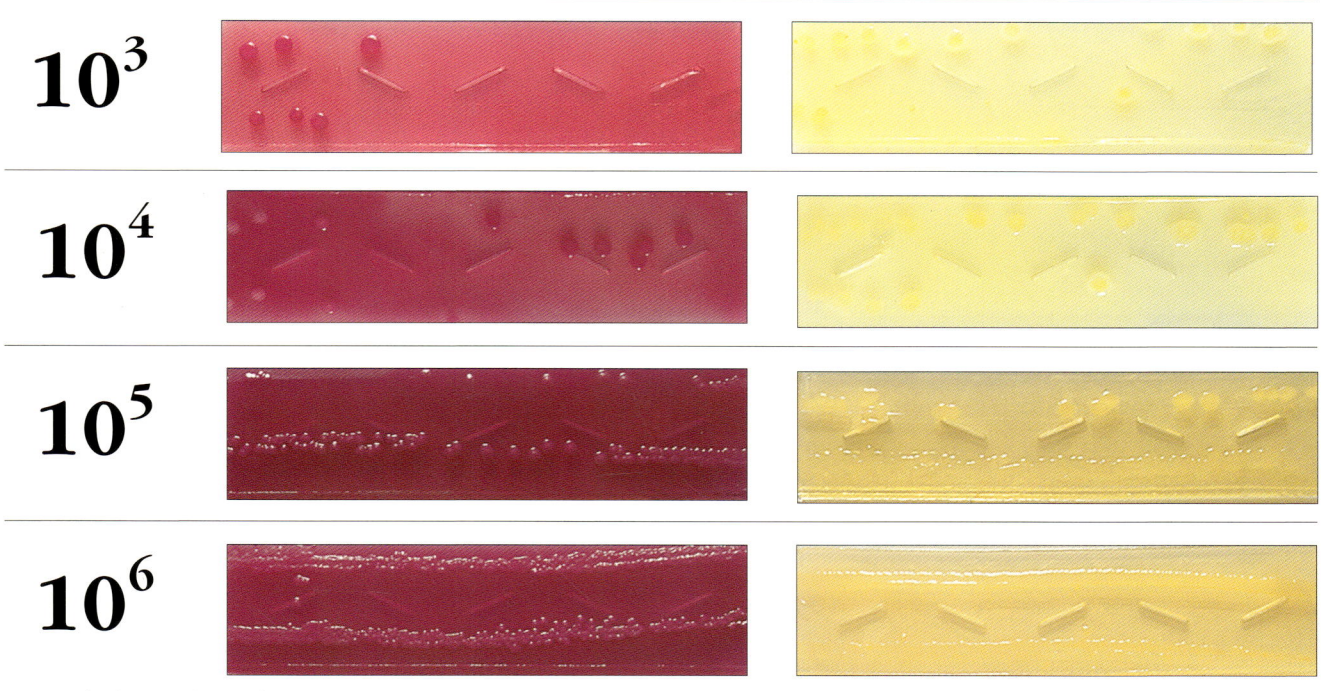
Bacterial concentration CFU/ml *	Number of visible colonies	
	CLED agar	MacConkey agar
10^3	5	4
10^4	17	15
10^5	110	100
10^6	~400	~400

**CFU/ml = Colony Forming Unit per 1ml of urine*

EXAMPLES OF RESULTS:

Concentration

CFU/ml *



** CFU/ml = Colony forming Unit per 1ml of urine*

CORRECT STORAGE AND SHELF LIFE:

Diaslide® is stored at Room Temperature (15-25°C) in the original package.

DIASLIDE PACKAGING:

Diaslide	Cat. No.	Packaging
CLED/MacConkey	DS-101	10x10 units
Columbia CNA/MacConkey	DS-102	10x10 units
Chromogenic/MacConkey	DS-105	10x10 units

Novamed Ltd.
28 Pierre Koenig Street
Talpiot Industrial Area
POB 53231
Jerusalem, Israel

Tel: 972-2-6781861
Fax: 972-2-6781852

e-mail: info@novamed.co.il
www.novamed.co.il

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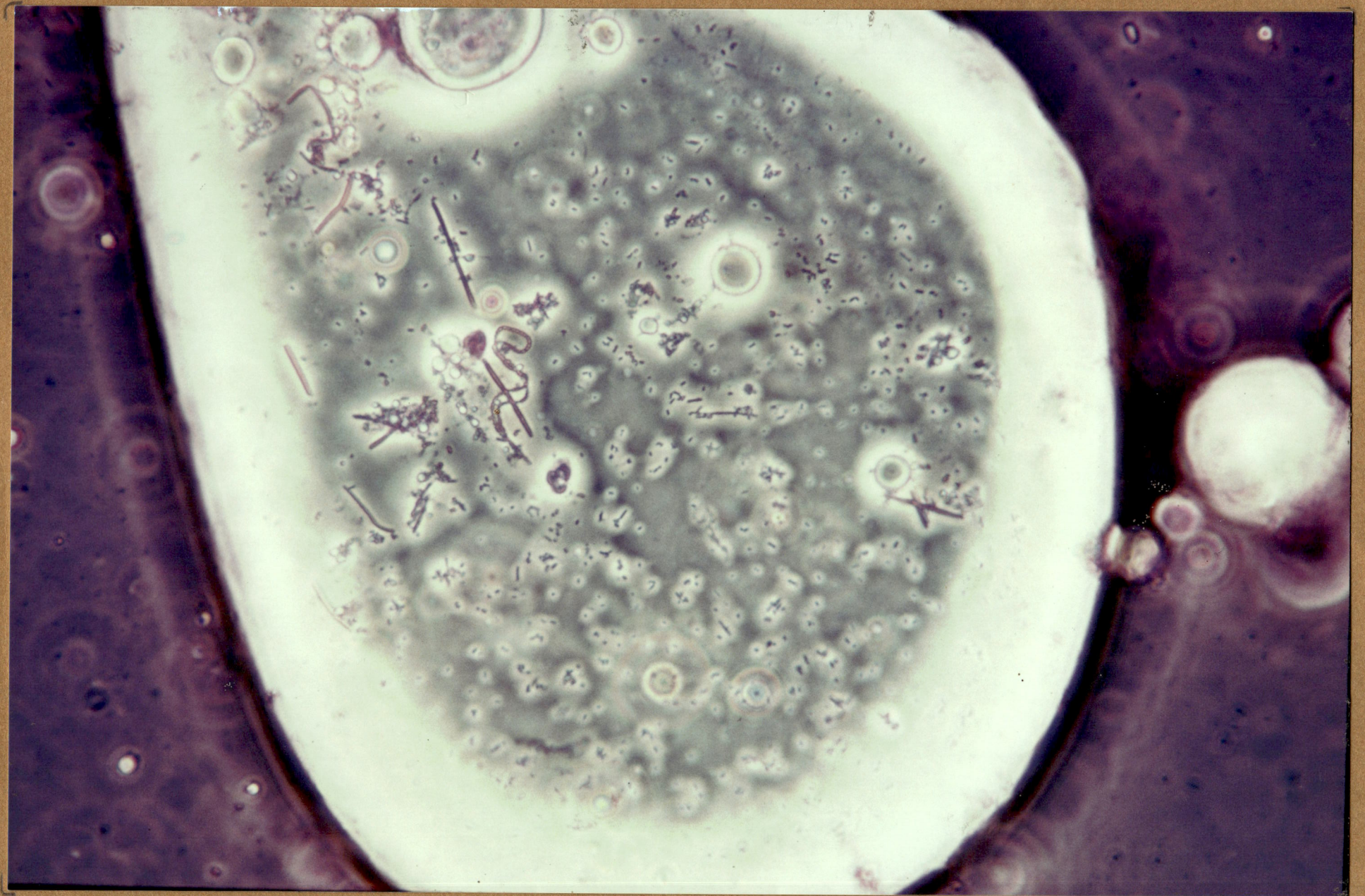

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Dentyl*pH*

Sure to Take Your Breath Away

A range of highly innovative products developed specifically for the prevention and treatment of bad breath. No other products work in the same way as Dentyl *pH*. They are technically advanced, unique and have been designed to play an important role in the oral hygiene routine.

Now, isn't *this* something your best friend *should* have told you about?

Dentyl *pH* Microgel
Ultimate Care Toothpaste
Action right where it matters

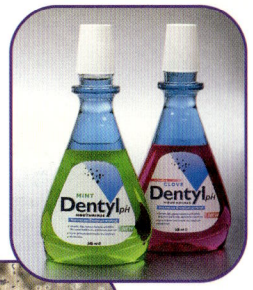
A scientifically advanced gel toothpaste containing micro-capsules filled with a most effective anti-bacterial agent. The soft capsules burst on brushing to deliver this agent direct to the areas that matter, combining with other complementary ingredients to ensure reduction in harmful bacteria and the *ultimate* in cleaning, whitening, protection and care.

- **Advanced anti-bacterial system** - 3 anti-bacterial agents, one of which is micro-encapsulated for maximum freshness and effectiveness
- **Ultimate care** - more than a total care toothpaste
- **Maximum protection** - helps fight cavities, plaque, tartar, gum & mouth complaints
- **A Whiter smile** - through powerful yet gentle cleaning and effective stain removal
- **Aloe vera** and 3 other agents for soothing, beneficial care
- **Dramatically reduces harmful bacteria** - promotes fresh breath confidence
- **Contains fluoride** - strengthens teeth
- **Same pH balance as saliva** - ideal for sensitive teeth and gums
- **Special low-froth formula** - enhances efficiency and reduces 'dribble-factor'

Dentyl *pH* Tongue Cleaner

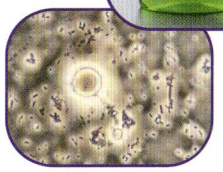
The smart way to a cleaner, better tasting mouth. Use as part of your oral hygiene routine, together with Dentyl *pH* Microgel toothpaste and Mouthrinse for the ultimate in fresh breath confidence.

- **New, unique curved shape** - fits the contour of the tongue
- **Dual-action head** - twice as effective as other designs
- **Removes bacteria**, food, debris, dead skin cells
- **Designed by the world's leading expert in breath odour**
- **Leaves mouth and breath fresh**



Dentyl *pH*
Mouthrinse
Seeing is believing

The only mouthrinse you can actually see working.



Hundreds of bacteria and debris which have been bound to a single drop of Dentyl pH. These are coloured by the formula and discarded in the rinse

Dentyl *pH* mouthrinse is a real breakthrough, the *only* mouthrinse of its kind. It absorbs, tints, lifts and physically removes oral bacteria and debris that cause bad breath while you swish and gargle, leaving them clearly visible in the sink after rinsing. The harmful elements are washed down the sink, not left in your mouth. The only mouthrinse that physically removes the bacteria and debris that cause bad breath - right before your eyes. You can actually see it working.

- **Long-lasting** - effective for up to 18 hours after a single use
- **Alcohol-free** - most mouthrinses contain alcohol, drying the mouth
- **Fluoride** - to strengthen teeth and prevent decay
- **Helps fight plaque** and protects teeth and gums
- **Same pH balance as saliva** unlike some mouthrinses which are acidic and can damage the mouth - Dentyl *pH* does not
- **Designed by the world's leading expert in breath odour**

Available from leading chemists, dentists and supermarkets. Dentyl *pH* Microgel ultimate care toothpaste and Mouthrinse are available in two pleasant flavours - mint and refreshing clove.

Dentyl *pH* Advice Service

If you have a question or problem relating to bad breath then please write, in confidence, to the **Dentyl *pH* Advice Service** at:
Fresh Breath Ltd, 2 Devonshire Place, London, W1N 1PA
or email at fresh.breath@virgin.net

Would your best friend really tell you?

How to achieve the ultimate in Fresh Breath Confidence

Bad Breath is an Extremely Common Problem

96% of the adult population suffer from bad breath at some time in their life, but most people periodically worry about it. The difficulty is of course, that bad breath is almost impossible to self-detect, so it's very easy to be oblivious to any problem. And, it's not something people will usually tell you about, either. After all, it's such a taboo subject, even our best friends are unlikely to want to tell us.

Well, would you really trust your best friend to tell you?

This leaflet explains what causes bad breath, it identifies some of the tell-tale signs and provides tips to ensure you enjoy the *ultimate* in fresh breath confidence.

Facing the Facts

the truth about bad breath

There are two ends of the spectrum with bad breath:

- *'Morning breath'* which many people suffer from. As our salivary glands rest while we sleep, there's insufficient saliva to wash away bacteria and food debris. It is, however, a temporary problem, which can be rectified simply by brushing and rinsing with an anti-bacterial alcohol-free mouthrinse.
- *Halitosis,* by contrast, is more serious and will need treatment. It is prolonged, often with more severe symptoms, caused by an excess of bacteria giving off odorous gases.

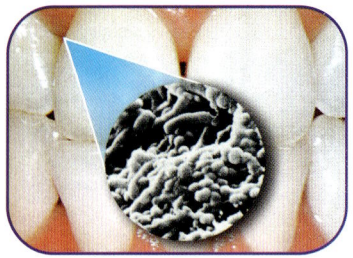
The Tell-Tale Signs

Of course, no-one knows whether their best friend would tell them the truth about their breath, so below are a couple of things to look out for which indicate bad breath problems: -

- **Gums that bleed on brushing or flossing, or recede**
- **A dry mouth**
- **An unpleasant, metallic taste**

If you have any of the above try the **'lick-wrist test'**. While it isn't conclusive, it is a way of discovering whether your breath could be letting you down: - *Poke your tongue out as far as possible and run your wrist down its length starting as far back as possible. If an odour is present on your wrist once the saliva has dried, then you are likely to be suffering from bad breath.*

What Causes Bad Breath ?



Bacteria and debris shelter between teeth

Bad breath is principally caused by excess bacteria which build up in the mouth as a result of inadequate oral hygiene. Thriving in the warm and oxygen-free environment, these bacteria feed on the cocktail of proteins, food debris and dead cells present in the mouth, leaving decay and gum disease in their wake and expelling foul-smelling gases.

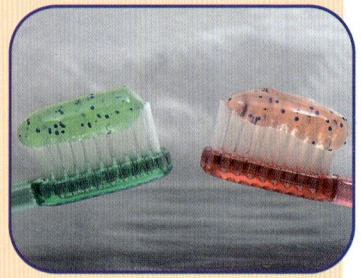
But, there are also many other factors, which can cause our breath to smell, such as:

- ***food breath*** - caused by what we eat. Avoided by omitting foods and liquids such as garlic, onion, fatty foods and alcohol
- ***hunger breath*** - caused by a lack of food and liquids as we 'meal-skip'. Overcome by ensuring we eat and drink plenty
- ***dental disease*** - normally resolved by a correct oral hygiene routine and visiting the dentist regularly
- ***illness*** - if we are suffering from systemic illnesses
- ***hormonal changes***
- ***stress***

Hints & Tips on How to Achieve Long Lasting Fresh Breath Confidence

It is important to ensure that excess bacteria and debris are removed from the mouth. No single oral hygiene technique is sufficient to remove them all - and bacteria are very tenacious - so to achieve the ultimate fresh breath confidence, it's vital to combine brushing and flossing with the use of a good mouthrinse.

A good oral hygiene routine using products that are specifically designed to combat and remove bacteria will lead to fresher breath: -



- Brush morning and evening moving around the mouth. Keep the toothbrush at an approximate 45° angle to the teeth and pay special attention to the area where gums and teeth meet.
- Use an advanced anti-bacterial toothpaste that works efficiently and directly at the problem area, such as Dentyl *pH* Microgel ultimate care toothpaste.
- A mouthrinse reaches places that brushing cannot, but make sure that it is alcohol-free, as alcohol can make the situation worse due to its drying effect. Most mouthrinses only temporarily disguise a problem by strong flavouring, but Dentyl *pH* physically absorbs, lifts and removes the dead bacteria and debris from the mouth.
- Using floss or tape, wood sticks or another form of interdental cleaning aid to reach between the teeth will greatly enhance the effects of your dental hygiene routine.
- A high percentage of bacteria accumulate on the tongue, so use a tongue cleaner as often as you can to ensure that your mouth is really clean. You'll find an easy to use tongue cleaner in the Dentyl *pH* range.
- Pay regular visits to your dentist or hygienist and ask them to check your cleaning technique.
- If you think you may have a bad breath problem contact a specialist such as The Fresh Breath Centre, London (020 7935 1666) where experienced, sensitive experts are available to help. Here, special tests are carried out to ascertain whether or not you have a problem and if so, how to treat it.

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